

What is claimed is:  
CLAIMS

10/18/01

- Sub C1 > 1. A peptide comprising at least 9 contiguous amino acids of SEQ.ID.NO.1.  
 2. A peptide comprising the amino acid sequence of SEQ.ID.NO.3 or a functional fragment thereof.

- Sub A2 > 3. A peptide according to claim 1 or 2 exhibiting trypanolytic activity preferably in combination with cytolytic and/or glucan binding and/or LPS binding and/or opsonizing activity.  
 4. An antibody specifically recognizing the peptide of any of the preceding claims or a fragment or epitope thereof.

- Sub C1 > 5. A DNA sequence encoding an Eisenia foetida protein or polypeptide or encoding an immunologically active and/or functional fragment thereof selected from the group consisting of  
 (a) DNA sequences comprising a nucleotide sequence encoding a protein or peptide comprising the amino acid sequence as given in SEQ ID NO. 1 or 3;  
 (b) DNA sequences comprising a nucleotide sequence as given in SEQ ID NO: 2;  
 (c) DNA sequences hybridizing with the complementary strand of a DNA sequence as defined in (a) or (b) and encoding an amino acid sequence which is at least 80% identical to the amino acid sequence encoded by the DNA sequence of (a) or (b);  
 (d) DNA sequences the nucleotide sequence of which is degenerated as a result of the genetic code to a nucleotide sequence of a DNA sequence as defined in any one of (a) to (c); and  
 (e) DNA sequences encoding a fragment of a protein encoded by a DNA sequence of any one of (a) to (d).

- Sub A3 > 6. A recombinant expression vector comprising a DNA sequence according to claim 5.

- Sub C1 > 7. A host cell transformed or transfected with an expression vector according to claim 6.

Sub A4> 8. The host cell of claim 7 wherein the host cell is selected from the group consisting of *E. Coli*, *Bacillus sp.*, *Streptomyces sp.*, yeast, fungi, insect cells, plant cells or mammalian cells.

Sub A5> 9. The host cell of claim 8, wherein the host cell is *E. Coli*.

10. A method for the production of an *Eisenia foetida* polypeptide or an immunologically active or functional fragment thereof comprising culturing a host cell of claim 7, 8 or 9 under conditions allowing the expression of said polypeptide and recovering the produced polypeptide from the culture.

11. A pharmaceutical composition comprising at least a peptide according to claim 1, 2 or 3.

Sub A6> 12. Use of a peptide according to claim 1, 2 or 3 for the preparation of a medicament to treat trypanosomal infection, bacterial infection or cancer.

Add A8>

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**Table 1 : aminoacid sequence of CCF-1 and TNF/TIP peptides**

| <b>Peptide</b>          | <b>Amino acid sequence</b>  |
|-------------------------|---|
| CCF-1.1                 | N-terminus : NH <sub>2</sub> -FTDWDQYHIVWQDEFDYFDGAKWQHEVTAT-COOH |
| CCF-1.2                 | (R,K) ↓ NH <sub>2</sub> -VYK-COOH                                 |
| CCF-1.4                 | (R,K) ↓ NH <sub>2</sub> -NTGGEFLGIQK-COOH                         |
| CCF-1.5                 | (R,K) ↓ NH <sub>2</sub> -MGSTMHWGPGWDDNER-COOH                    |
| CCF-1.8                 | (R,K) ↓ NH <sub>2</sub> -YWLTS LPK-COOH                           |
| CCF-1.10<br>(CCF-1/TIP) | (R,K) ↓ NH <sub>2</sub> -SGEIDI IETIGNR-COOH                      |
| TNF/TIP                 | TPEGAEA   |

**Table 2 : trypanolytic activity of CF and CCF-1.**

| CF tested <sup>a</sup>                                     | Neutralizing antibody <sup>d</sup><br>(12C9) | % Trypanolysis | % Inhibition |
|--|--|----------------|--------------|
| 1. Total CF <sup>b</sup>                                   | -  | 97             |              |
|  | +  | 10             | 90           |
| 2. CF flow through <sup>b</sup><br>(irrelevant IgG column) | -  | 94             |              |
|  | +  | 7              | 93           |
| 3. CF flow through <sup>b</sup><br>(12C9 column)           | -  | 30             |              |
|  | +  | 2              | 94           |
| 4. Eluate (CCF-1) <sup>c</sup><br>(12C9 column)            | -  | 42             |              |
|  | +  | 0              | 100          |

a : CF and CF subfractions were purified by immunoaffinity on irrelevant IgG or 12C9 column and tested for trypanolytic activity in the trypanolysis assay (% trypanolysis was recorded after 2 hrs).

b : Concentration used = 1 mg/ml.

c : Concentration used = 4 µg/ml.

d : 12C9 antibody was added at a concentration of 10 µg/ml.

**Table 3 : inhibition of the trypanolytic activity (*T. brucei*) of CCF-1 and TNF- $\alpha$  by antibodies and carbohydrates**

| Inhibitor <sup>a</sup>      | CCF-1 mediated trypanolysis <sup>b</sup> |              | TNF- $\alpha$ mediated trypanolysis <sup>c</sup> |              |
|-----------------------------|--|--------------|--|--------------|
|                             | % Lysis                                  | % Inhibition | % Lysis  | % Inhibition |
| None                        | 42                                       | -            | 41   | -            |
| N,N-diacetylchitobiose      | 3  | 73           | 0  | 100          |
| Cellobiose                  | 49                                       | 0            | 41   | 0            |
| Polyclonal anti-TNF/TIP     | 0  | 100          | 0  | 100          |
| Polyclonal IgG control      | 46                                       | 0            | 43   | 0            |
| Monoclonal anti-TNF/TIP     | 0  | 100          | 0  | 100          |
| Monoclonal IgG control      | 49                                       | 0            | 41   | 0            |
| Monoclonal anti-CCF-1(12C9) | 0  | 100          | 1  | 98           |
| Monoclonal anti-TNF(1F31F3) | 44                                       | 0            | 41   | 0            |

a : Inhibitors were added at a final concentration of 10  $\mu$ g/ml.

b : CCF-1 was added in the trypanolysis assay at a final concentration of 4  $\mu$ g/ml.

c : TNF- $\alpha$  was added in the trypanolysis assay at a final concentration of 1.000 U/ml.

**Table 4 : inhibition of the trypanolytic activity (*T. cruzi*) of CF by antibodies and carbohydrates**

| Inhibitor <sup>a</sup>      | CF-1 mediated trypanolysis <sup>b</sup> |              |
|-----------------------------|---|--------------|
|                             | % Lysis                                 | % Inhibition |
| None                        | 62                                      | -            |
| N,N'-diacetylchitobiose     | 19                                      | 70           |
| Cellobiose                  | 67                                      | 0            |
| Monoclonal anti-CCF-1(12C9) | 30                                      | 52           |
| Monoclonal IgG control      | 67                                      | 0            |

a : Inhibitors were added at a final concentration of 10 µg/ml.

b : CF was added in the trypanolysis assay at a final dilution of 1 : 4.000.

**Table 5 : inhibition of the cytolytic activity of CCF-1 (L929) by antibodies and carbohydrates**

| Inhibitor <sup>a</sup>      | CCF-1 mediated cytotoxicity <sup>b</sup> |              |
|-----------------------------|--|--------------|
|                             | % Lysis                                  | % Inhibition |
| <u>Experiment 1</u>         |  |              |
| None                        | 72                                       | -            |
| N,N'-diacetylchitobiose     | 0  | 100          |
| Monoclonal anti-CCF-1(12C9) | 0  | 100          |
| Monoclonal anti-TNF/TIP     | 0  | 100          |
| <u>Experiment 2</u>         |  |              |
| None                        | 66                                       | -            |
| Monoclonal anti-CCF-1(12C9) | 14                                       | 79           |
| Monoclonal anti-CCF-1(7F1)  | 0  | 100          |
| Monoclonal anti-CCF-1(6H1)  | 0  | 100          |

a : Inhibitors were added at a final concentration of 10 µg/ml

b : CCF-1 was added in the L929 cytotoxicity assay at a final concentration of 4 µg/ml

**Table 6 : parasitaemia in mice treated with anti-CCF-1 mAbs (group of 10 mice)**

| Day pi | Parasites x 10 <sup>6</sup> /ml |                    |
|--------|---------------------------------|--------------------|
|        | Control mAb-treated             | anti-CCF-1 treated |
| 3      | 104                             | 135                |
| 4      | 129                             | 194                |
| 5      | 64                              | 84                 |
| 6      | 2                               | 2                  |



**Table 7 : parasitaemia in untreated or CCF-1-treated mice (group of 4 mice)**

| Day pi | Parasites x 10 <sup>6</sup> /ml |                |
|--------|---------------------------------|----------------|
|        | untreated                       | rCCF-1 treated |
| 3      | 207                             | 142            |
| 4      | 211                             | 143            |
| 5      | 102                             | 104            |
| 6      | 6                               | 1.2            |

[illegible]

**Table 8: Production of TNF- $\alpha$  by 2C11-12 activated with CCF-1**

| $\mu\text{g/ml}$ CCF-1 | $\text{pg/ml}$ TNF- $\alpha$ |
|------------------------|------------------------------|
| 40                     | 5843                         |
| 20                     | 2483                         |
| 10                     | 1112                         |
| 5                      | 370                          |
| 2.5                    | 60                           |
| 1.25                   | 17                           |
| 0.625                  | Nd                           |

nd: not detectable

**Table 9: Production of TNF- $\alpha$  by C3H/J PECs activated with CCF-1**

| $\mu\text{g/ml}$ CCF-1 | $\text{pg/ml}$ TNF- $\alpha$ |                 |
|------------------------|------------------------------|-----------------|
|                        | - IFN- $\gamma$              | + IFN- $\gamma$ |
| 40                     | nd                           | 300             |
| 20                     | nd                           | 130             |
| 10                     | nd                           | 30              |
| 5                      | nd                           | Nd              |
| 2.5                    | nd                           | Nd              |
| 1.25                   | nd                           | Nd              |
| 0.625                  | nd                           | Nd              |

nd: not detectable